QUANTITATIVE CRITERIA OF ANTAGONISM

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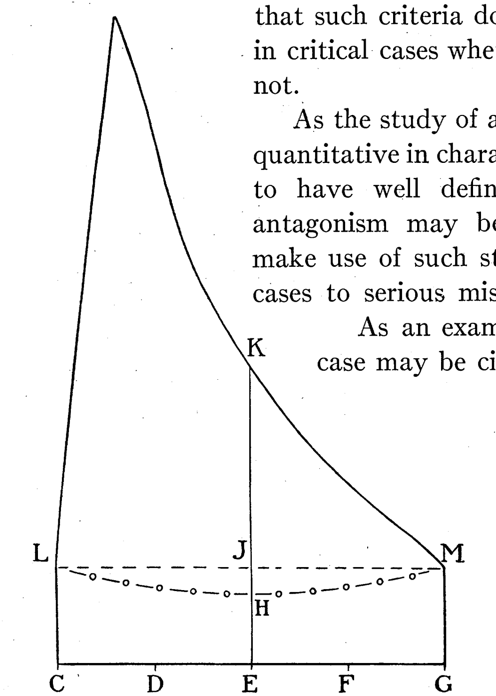
(WITH FOUR FIGURES)

It is apparent from a consideration of the literature that faulty criteria of antagonism are frequently employed. It often happens that such criteria do not permit us to decide in critical cases whether antagonism exists or not.

As the study of antagonism becomes more quantitative in character it becomes necessary to have well defined standards by which antagonism may be measured. Failure to make use of such standards has led in some cases to serious misconceptions.

As an example of this the following case may be cited. Suppose a solution of a toxic salt, A, to be mixed with a solution of another salt, B, the solutions having the same molecular concentration and the same degree of toxicity. Suppose that in a mixture of 100 cc. of 0.1 M solution of A plus 100 cc. of 0.1 M solution of B, plants grow better than in either of the pure solutions. Some investigators assert that this increase of growth should not be attributed wholly to



A	100	75	50	25	0 %
B	0	25	50	75	100 %

FIG. 1.—Curves showing the growth of roots in mixtures of equally toxic solutions of two salts A and B: the ordinates represent growth; the abscissas represent the composition of the mixtures, thus A 50, B 50 means a mixture in which the dissolved molecules are 50 per cent A and 50 per cent B; the horizontal dotted line (LJM) represents the growth which would occur if there were no antagonism (additive effect); LKM is the antagonism curve; LHM, curve expressing increased toxicity (opposite of antagonism); the quantitative expression of antagonism at the point E is $\frac{KJ}{JE}$.

antagonistic action, but that it is partly due to the dilution of the toxic salts, for it is evident that the concentration of each of them has been reduced from 0.1 M to 0.05 M.

It seems desirable to formulate the matter so as to obviate such misconceptions and it is clearly necessary to provide a quantitative criterion of antagonism which shall be both accurate and convenient. A graphical expression of such a criterion is seen in Fig. 1.

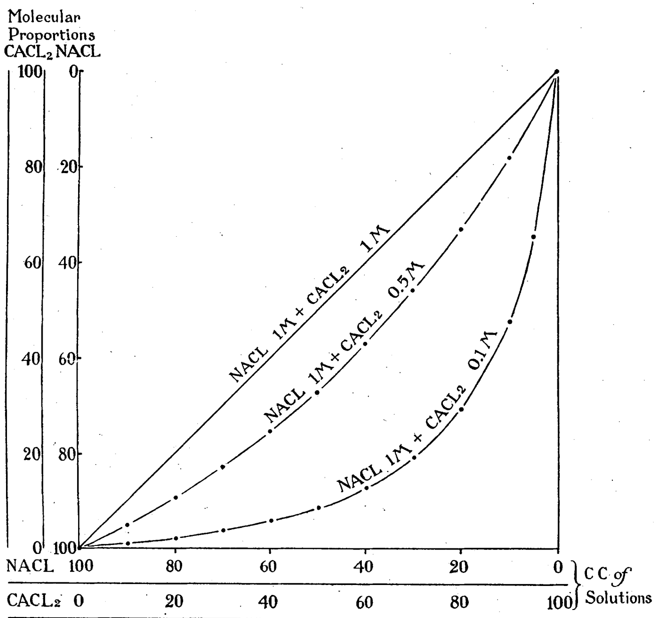


FIG. 2.—Curves showing the relation between the molecular composition of a mixture of two solutions and its composition expressed as cc. of the component solutions; see footnote 1.

In this figure the growth of the plant in a solution of the salt *A* 0.1 M is expressed by the ordinate at *C*; the growth in a solution of the salt *B* 0.1 M is expressed by the ordinate at *G*; as the ordinates are equal the solutions are equally toxic. The ordinates between *C* and *G* express the growth in various mixtures of the two solutions; thus that at *D* expresses the growth in a mixture in which 75 per cent of the dissolved molecules are *A* and 25 per cent

are *B;* the ordinate at *E* expresses growth in a mixture in which 50 per cent of the dissolved molecules are *A* and 50 per cent are *B*.[1]

It is obvious that the effect of mixing two equally toxic solutions must fall into one of the following categories.

1. The toxicity is unaltered, that is, the toxic action of the two salts is *additive*. Each salt produces its own toxic effect precisely as though the other were not present. This is expressed by the horizontal dotted line *LJM*.

It is evident that we cannot get increased growth by mixing two such solutions unless the salts have an antagonistic action. The dilution of *A* from 0.1 M to 0.05 M is exactly compensated by the introduction of molecules of *B*. Or, to put it in another way, the toxic effect depends on the number of molecules present (if both kinds of molecules are equally toxic and there is no antagonism) and it makes no difference whether the solutions are pure or mixed.

If the toxic effect depends on ions, rather than on molecules, then, since the number of ions may be somewhat increased by mixing solutions, the toxicity may be correspondingly increased; but the amount of this increase would ordinarily be negligible.

2. The toxicity is diminished, that is, the effect is *antitoxic*. We then get a curve rising somewhere above the dotted line, such as the unbroken line *LKM*.

3. The toxicity is increased. We then get a curve which somewhere falls below the dotted line, such as the line interrupted by circles *LHM*.

The considerations here set forth apply in all cases where two equally toxic solutions are mixed, whether their concentration is the

[1] In order to avoid unnecessary calculations in making up solutions with the desired molecular proportions, curves similar to those in fig. 2 serve a very useful purpose. The figures on the vertical scale denote molecular proportions, while those on the horizontal scale denote cc. of solutions. Suppose that we are mixing NaCl 1 M and $CaCl_2$ 0.5 M. If we mix 60 cc. of the NaCl with 40 cc. of the $CaCl_2$, the molecular proportions are NaCl 75 per cent+$CaCl_2$ 25 per cent. If each of the scales (vertical and horizontal) is 100 mm. long, the ordinate (measured from above downward) will in this case be 25 mm., and the abscissa (measured from right to left) 60 mm. After determining a series of such points, a curve may be drawn from which other proportions may be read off directly. Such a curve is shown in the figure (NaCl 1 M – $CaCl_2$ 0.5 M); this curve serves equally well for all solutions in which the molecular concentration of one component is twice that of the other. In the same manner the other curve NaCl 1 M – $CaCl_2$ 0.1 M applies equally well to all solutions in which the molecular concentrations of the two components are as one to ten.

same or not. Thus, if a solution of A 0.05 M is just as toxic as a solution of B 0.1 M, mixtures of the two will give a straight line (as in fig. 1) provided their effects are additive.

Emphasis should be laid upon the fact that the method of mixing two equally toxic solutions eliminates disturbances due to variations of osmotic pressure. If a molecule of A is twice as toxic as a molecule of B, a solution of A 0.05 M will be just as toxic as a solution of B 0.1 M, provided there are no other factors to be considered. But if the osmotic pressure of the 0.05 M solution of A is less than that of the 0.1 M solution of B, there will in many cases be better growth in the 0.05 M solution of A. In order to make the solution of A appear equally toxic with the solution of B, the concentration of A must be somewhat increased, say to 0.055 M. We thus compensate for the variation in osmotic pressure, and this compensation is not destroyed when the 0.055 M solution of A is mixed with the 0.1 M solution of B. If the effects of the salts are additive, we must therefore get a straight line, as shown in fig. 1.

It is evident that this straight line furnishes a criterion of antagonism which for quantitative purposes leaves nothing to be desired. All that is necessary is to determine what concentrations of A and B are equally toxic, mix these solutions in various proportions, and determine the amount of growth. The antagonism in any mixture may then be expressed in a very simple manner. Let the curve showing the growth in the mixtures be LKM. The antagonism in a mixture in which the molecules are 50 per cent A and 50 per cent B may be expressed as $\frac{KJ}{JE}$. JE is the growth which would have been obtained if the effect of the salts had been additive (that is, if there had been no antagonism, but each salt had produced its effect independent of the other). KJ is the increased growth due to antagonism; it is best expressed as percentage of JE or as $\frac{KJ}{JE}\times 100$.

In the same way increased toxicity (when the mixture is more toxic than either of the pure solutions) may be expressed as $\frac{JH}{JE}$. This sometimes occurs, but it is much less common than antagonism.

As an illustration of this method the results given in table I may be cited. In this case the growth in the various mixtures was in part determined directly and in part was calculated from results obtained by growing plants in mixtures having almost the same composition as the solutions given in the table.

TABLE I

MIXTURES OF EQUALLY TOXIC SOLUTIONS

Wheat (growth during 30 days) (NaCl 0.12 M + $CaCl_2$ 0.164 M)

Culture solution	Aggregate length of roots per plant in mm.	Additive effect	Antagonism
$CaCl_2$	55	55	....
75 per cent $CaCl_2$ 25 " NaCl	105	55	$\frac{105-55}{55}=0.91$
50 " $CaCl_2$ 50 " NaCl	180	55	$\frac{180-55}{55}=2.27$
25 " $CaCl_2$ 75 " NaCl	298	55	$\frac{298-55}{55}=4.42$
15 " $CaCl_2$ 85 " NaCl	370	55	$\frac{370-55}{55}=5.73$
5 " $CaCl_2$ 95 " NaCl	435	55	$\frac{435-55}{55}=6.91$
1 " $CaCl_2$ 99 " NaCl	300	55	$\frac{300-55}{55}=4.45$
NaCl	55	55	....

The percentages refer to molecular proportions; that is, 75 per cent $CaCl_2$+25 per cent NaCl means a solution in which 75 per cent of the dissolved molecules are $CaCl_2$ and 25 per cent are NaCl.

We may now consider the effect of mixing two solutions which are not equally toxic. Suppose solution *A* 0.1 M to be twice as toxic as solution *B* 0.1 M. The effect of mixing these, if the effects were strictly additive, would be the same as mixing a solution of *A* 0.1 M with another solution of *A* just twice as toxic, or in other words would be the same as increasing the concentration of *A*. In this case the curve expressing purely additive effects would not be a straight line, but would assume the form of a curved line, convex to the horizontal axis, similar to *VSW* in fig. 3. This is evident from the curves given by MAGOWAN[2] showing growth in toxic solutions of various concentrations.

[2] BOT. GAZ. 45:45. 1908.

It would be possible to determine this additive curve experimentally, and then to express antagonism quantitatively; for example, at the point P it would be expressed as $\frac{UT}{TP}$. But the labor would be much greater than by the method of mixing equally toxic solutions. The additive curve would be determined by growing plants, not in mixtures of A with B, but in mixtures of A with another solution of A having the same toxicity as B. Or we might use mixtures of B with another solution of B having the same toxicity as A. The two methods might not give exactly the same result. This is an additional argument in favor of using equally toxic solutions.

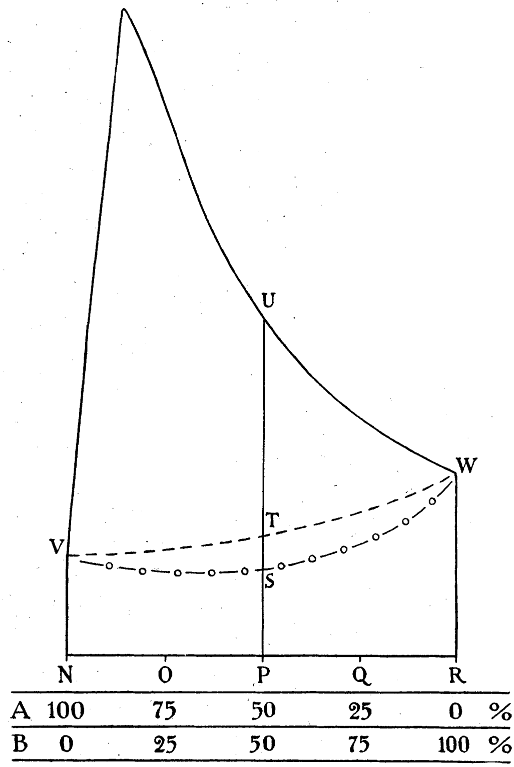


Fig. 3.—Curves showing growth in mixtures of unequally toxic solutions: the ordinates express growth; the abscissas express the composition of the mixtures as in fig. 1; the dotted line VTW expresses the growth which would occur if there were no antagonism (additive effect); VUW, antagonism curve; VSW, curve expressing increased toxicity (opposite of antagonism); the quantitative expression of antagonism at the point P is $\frac{UT}{TP}$.

An illustration of this method is found in the results given in table II. The growth in the various mixtures (additive and antagonistic) was in part determined directly and in part was calculated from results obtained by growing plants in mixtures

having almost the same composition as the solutions given in the table mentioned.

For the sake of completeness it may be mentioned that other types of antagonism curves are found; for example, flat-topped curves and also curves with two maxima, as shown in fig. 4.

If instead of mixing two equally toxic solutions we keep the concentration of one salt constant while varying that of the other, it becomes very difficult to determine the additive curve, especially

TABLE II

MIXTURES OF UNEQUALLY TOXIC SOLUTIONS

Wheat (growth during 30 days) ($NaCl$ 0.12 M + $CaCl_2$ 0.12 M)

Culture solution	Aggregate length of roots per plant in mm.	Additive effect	Antagonism
$CaCl_2$	85	85	....
75 per cent $CaCl_2$ 25 " $NaCl$	125	75	$\frac{125-75}{75}=0.67$
50 " $CaCl_2$ 50 " $NaCl$	195	66.5	$\frac{195-66.5}{66.5}=1.93$
25 " $CaCl_2$ 75 " $NaCl$	310	60	$\frac{310-60}{60}=4.17$
15 " $CaCl_2$ 85 " $NaCl$	380	58	$\frac{380-58}{58}=5.55$
5 " $CaCl_2$ 95 " $NaCl$	438	56	$\frac{438-56}{56}=6.82$
1 " $CaCl_2$ 99 " $NaCl$	300	55	$\frac{300-55}{55}-4.45$
$NaCl$	55	55	....

The percentages refer to molecular proportions; that is, 75 per cent $CaCl_2$+25 per cent $NaCl$ means a solution in which 75 per cent of the dissolved molecules are $CaCl_2$ and 25 per cent are $NaCl$.

when variations in osmotic pressure influence the result. It is therefore difficult to obtain an accurate quantitative expression of antagonism by this method, and in critical cases it may be impossible to decide whether antagonism exists or not.

Emphasis should be laid on the fact that the growth of parts not in immediate contact with the solution does not furnish a trustworthy criterion of antagonism. Thus the leaves of wheat (which are not in contact with the solution) often grow well at the start in

solutions of copper salts, while the roots (which are immersed in the solution) do not grow at all. The reason is that as the solution passes through the roots to the leaves a great part of the copper is removed either by adsorption or by chemical combination.[3]

Finally, it may be noted that quantitative results are more reliable and much more easily obtained when uniform material is used. "Pure line" wheat and other seeds may now be obtained and should be used whenever possible. When they are not obtainable the following method (suggested by Professor JOHANNSEN) may be employed. Several heads of wheat are taken. One grain of wheat from each is placed in each solution (and these grains should resemble each other as much as possible). In this way each solution receives the same kinds of wheat, and an average of the growth of all the plants in any solution may be safely used for comparison with the average in any other solution. It is desirable to employ this method even when pure line seeds are used.

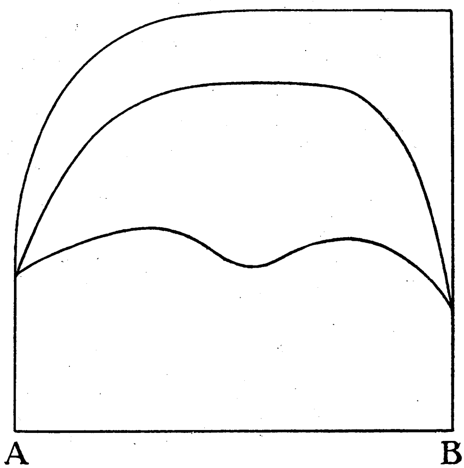


FIG. 4.—Types of antagonism curves: the ordinates express growth; the abscissas express the composition of the mixtures as in fig. 1.

Summary

The method of mixing equally toxic solutions furnishes the best criterion of antagonism, since we know at the outset just what effect each mixture must have provided there is no antagonism.

Mixtures of two equally toxic solutions must have precisely the same effect on growth as the pure solutions themselves, provided that the effects of the salts are additive. If antagonism exists there

[3] Cf. BOT. GAZ. 44:268. 1907.

is an increased growth in the mixtures. The amount of this increase, expressed as percentage of the growth obtained in the pure solutions, is the most satisfactory measure of antagonism.

The most reliable results are obtained by the use of uniform material and by taking for measurement only such parts as come into immediate contact with the solution.

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